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Application of ^{1}H NMR-based serum metabolomic studies for monitoring female patients with rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis is a chronic autoimmune-based inflammatory disease that leads to progressive joint degeneration, disability, and an increased risk of cardiovascular complications, which is the main cause of mortality in this population of patients. Although several biomarkers are routinely used in the management of rheumatoid arthritis, there is a high demand for novel biomarkers to further improve the early diagnosis of rheumatoid arthritis, stratification of patients, and the prediction of a better response to a specific therapy.

In this study, the metabolomics approach was used to provide relevant biomarkers to improve diagnostic accuracy, define prognosis and predict and monitor treatment efficacy. The results indicated that twelve metabolites were important for the discrimination of healthy control and rheumatoid arthritis. Notably, valine, isoleucine, lactate, alanine, creatinine, GPC APC and histidine relative levels were lower in rheumatoid arthritis, whereas 3-hydroxyisobutyrate, acetate, NAC, acetoacetate and acetone relative levels were higher. Simultaneously, the analysis of the concentration of metabolites in rheumatoid arthritis and 3 months after induction treatment revealed that L1, 3-hydroxyisobutyrate, lysine, L5, acetoacetate, creatine, GPC + APC, histidine and phenylalanine were elevated in RA, whereas leucine, acetate, betaine and formate were lower. Additionally, metabolomics tools were employed to discriminate between patients with different *IL-17A* genotypes.

Metabolomics may provide relevant biomarkers to improve diagnostic accuracy, define prognosis and predict and monitor treatment efficacy in rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune-based inflammatory disease that leads to progressive joint degeneration, disability, and an increased risk of cardiovascular complications, which is the main cause of mortality in this population [1]. The etiopathogenesis of RA is multifactorial and not fully known, which is characteristic of most autoimmune diseases. An improved understanding of RA etiopathogenesis and immunological disorders has led to modern therapeutic options, including TNF-α inhibitors. Although therapy with TNF-α inhibitors constitutes a breakthrough in RA management, no improvement is achieved in approximately 30% of cases, and another 20% of patients discontinue therapy because of side effects. There is also an ongoing search for biochemical and clinical markers that would allow the prediction of a good response to therapy with biologicals, including TNF-α inhibitors. Moreover, the results of our recent studies

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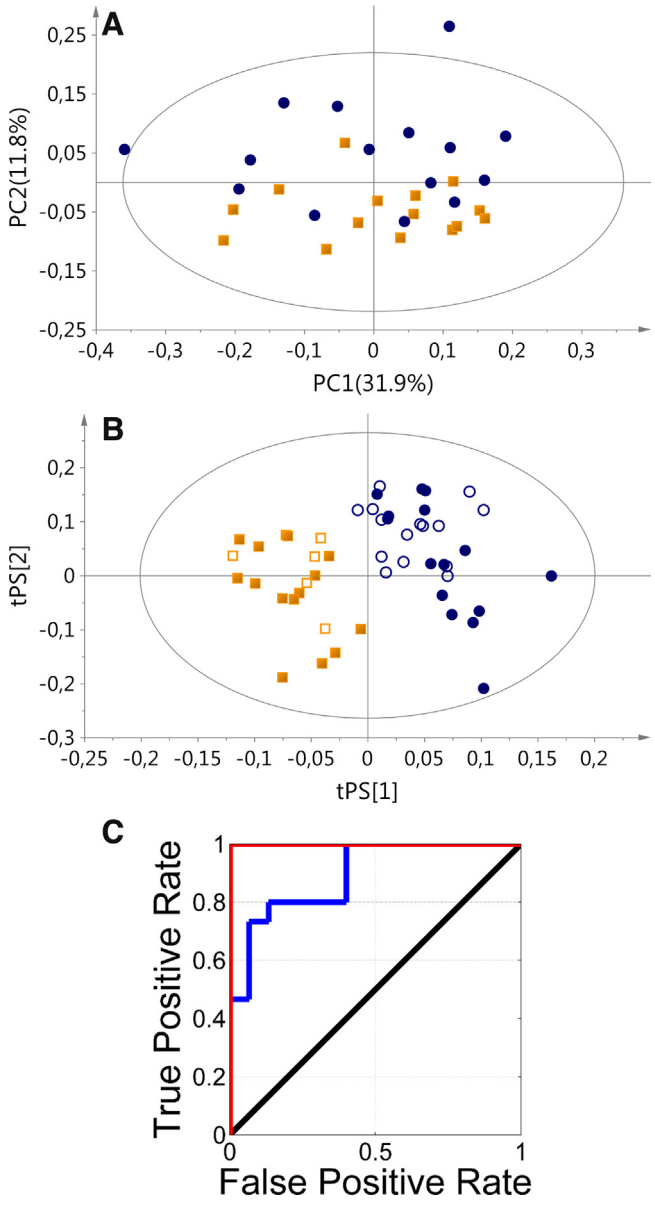

Fig. 1. The PCA model ((A); $R^2X_{cum} = 0.437$, $Q^2_{cum} = 0.272$) and PLS-DA model ((B); $R^2X_{cum} = 0.551$, $Q^2_{cum} = 0.761$) with the ROC curve (C) for comparison of the HC and RA groups. Blue circles—healthy controls; yellow boxes—patients before anti-TNF-α treatment. Empty symbols—the prediction set; solid symbols—the model set. (For interpretation references to color in this figure legend, the reader is referred to the web version of this article.)

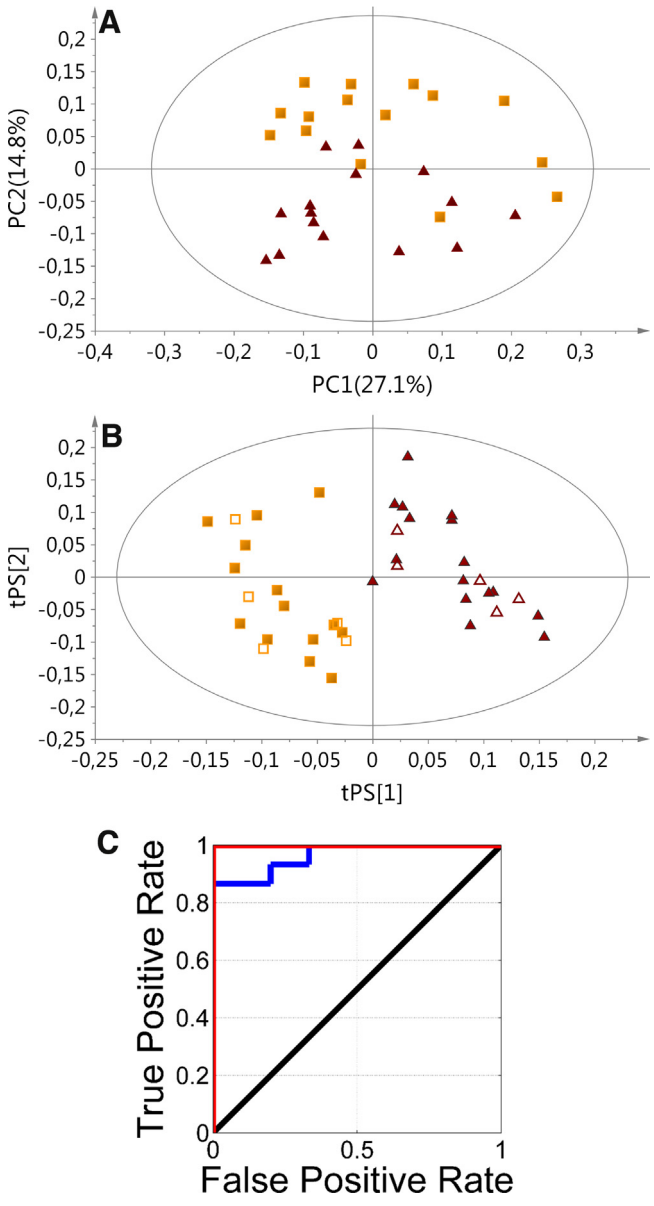

Fig. 2. The PCA model ((A); $R^2X_{cum} = 0.513$, $Q^2_{cum} = 0.351$) and PLS-DA model ((B); $R^2X_{cum} = 0.475$, $Q^2_{cum} = 0.767$) with the ROC curves (C) for comparison of the RA and RAT groups. Yellow boxes—patients before treatment; red triangles—patients after 3 months of anti-TNF-α treatment. Empty symbols—the prediction set; solid symbols—model set. (For interpretation references to color in this figure legend, the reader is referred to the web version of this article.)

have suggested that genetic variations within genes encoding for factors involved in inflammatory processes associated with RA development may play a significant role in disease susceptibility, progression and response to anti-TNF-α treatment [2,3]. In addition to clinical factors, genetic predisposition in combination with metabolomics may be helpful in clinical predictions. Although several biomarkers are routinely used in the management of RA, there is a high demand for novel biomarkers to further improve the early diagnosis of RA, the stratification of patients, and the prediction of a better response to specific therapies. In addition to genomics and proteomics, the implementation of metabolomic techniques may also improve our knowledge of the etiopathology of RA [4], as shown in our previous work in patients with inflammatory bowel disease [5] and pulmonary disease [6].

The present study assessed the potential impact of the application of metabolomic studies in patients with RA to determine whether metabolomic biomarkers of disease diagnostics and disease activity can be identified.

2. Materials and methods

2.1. Study population/characteristics of RA patients

The study included 20 Caucasian women patients meeting the EULAR/ACR (European League Against Rheumatism/American College of Rheumatology) 2010 criteria for (RA) and 30 healthy female volunteers (HC) as the control group. The patient characteristics are presented in Table 1. There was no statistically significant difference in sex and age between the populations.

Seventy serum samples were collected from adult individuals (women), including 40 samples obtained from 20 patients before

Table 1
Demographic data and clinical profiles of patients included in the study.

Feature	Value
RA patients (*N*=)	20
Age (years)	54 ± 12.3 (range: 28–74)
Females (%)	100
Disease duration (years)	12 ± 8.26 (range 3–25)
Disease onset (years)	42 ± 12.1 (range 17–65)
Current smokers (%)	18
RF+ (%)	80
ACPA+ (%)	85
DAS28 at baseline	6.84 ± 0.63 (range 5.24–8.05)
DAS28 at week 12 of anti-TNF drug	4.22 ± 1.10 (range 2.1–5.31)
Etanercept (%)	80
Adalimumab (%)	20
Glucocorticoids (%)	90 (mean dose 8 mg prednisone daily)
Methotrexate (%)	90 (mean dose 20.6 mg weekly)

RA—rheumatoid arthritis; RF—rheumatoid factor; ACPA—anti-citrullinated protein antibody; DAS28—disease activity score in 28 joints.

and after 3 months of treatment (RAT) and 30 control serum samples collected from healthy individuals.

The female patients enrolled in the study were ≥18 years of age with active, adult-onset RA. The mean disease duration was 12 (range: 3–25) years. All the patients provided written informed consent. The study was approved by the Wroclaw Medical University Ethics Committee (No. KB 577/2011, 10.11.2011).

The following inclusion criteria were accepted: consent to participate in the study; active form of the disease–disease activity score based on erythrocyte sedimentation rate and an evaluation of 28 joints (DAS28) ≥5.1; failure of treatment with at least 2 csDMARDs; over 18 years of age; women with reproductive potential were required to use reliable contraception.

The following exclusion criteria were applied: pregnancy or breastfeeding; coexistence of other systemic diseases of connective tissue besides RA; clinically significant impairment of hepatic and renal function; alcohol abuse; infection with hepatotropic viruses; infections resistant to therapy; ongoing history of cancer if no cure was achieved; uncontrolled diabetes; and patient unwilling or unable to cooperate.

The patients were administered the recommended doses of TNF-α inhibitors (etanercept [ETA] or adalimumab [ADA]) as follows: subcutaneous injection of ADA at 40 mg every other week and subcutaneous injection of ETA at 50 mg every week. The patients were allowed to continue treatment with csDMARDs, glucocorticoids or non-steroidal anti-inflammatory drugs if the treatment regimens were not modified for 4 weeks before the study. In total, 90% of patients were treated with a stable dose of methotrexate (mean dose: 20.6 mg weekly), and 90% patients were treated with glucocorticoids (mean dose: 8 mg daily of prednisone). To examine the response to anti-TNF therapy in RA, blood samples, laboratory data, and clinical data were collected at baseline (prior to anti-TNF therapy) and at 3 months after treatment. The clinical evaluation was based on medical history, number of painful and swollen joints, pain intensity assessed by the patient on a 100-mm visual analogue scale (VAS) and laboratory tests (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP]), blood cell count, aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, serum creatinine, urea levels and urinalysis. The parameters allowed for the determination of improvement according to the criteria based on DAS28 as suggested by the EULAR. According to the EULAR definition, the patients were classified as good, moderate, or non-responders, using the individual amount of change in the DAS28 (ΔDAS28) and DAS28 values at 3. Moreover, in some analyses, a comparison was made between non-responders versus patients in remission or low disease activity according to the EULAR response criteria. This stratification was due to the limited number of patients in each group.

2.2. IL-17A genotyping

The biallelic polymorphism within the *IL-17A* gene (rs2275913; G-197A) was studied as previously described by Bogunia-Kubik et al. [2]. In brief, DNA was extracted from peripheral blood taken on EDTA using the Maxwell 16 Blood DNA Purification Kit (Promega Corp., Madison, WI, USA) following the recommendations of the manufacturer. The *IL-17A* (rs2275913; G-197A) alleles were determined by real-time polymerase chain reaction (PCR) amplifications with the use of the LightSNiP assay designed by TIB MOLBIOL (GmbH, Berlin, Germany). The reaction was performed following the recommendation of the manufacturer, and the typing results were analyzed using a Roche LightCycler 480 instrument.

2.3. Sample preparation for proton NMR spectroscopy

Serum was sampled from the peripheral vein and centrifuged for 10 min at 4000 × *g*. The samples were frozen in liquid nitrogen immediately after collecting and stored at −80 °C until analysis.

Prior to the metabolomic experiment, the serum samples were thawed at room temperature and vortexed. Next, the mixtures of 200 μL of serum and 400 μL of saline solution (prepared from 0.9% NaCl, 15% D_2O and 3 mM TSP) were mixed again. After centrifugation (12 000 × *g*, 10 min), an aliquot of 550 μL of each sample supernatant was subsequently transferred to a 5 mm NMR tube. The samples were kept at 4 °C before measurement.

2.4. ^1H NMR measurements

The NMR spectra of serum samples were recorded at 300 K using an Avance II spectrometer (Bruker, GmBH, Germany) operating at a proton frequency of 600.58 MHz. The NMR spectra of the serum samples were recorded using a CPMG pulse sequence with water presaturation in the Bruker notation. For each sample, 128 scans were collected with a spin-echo delay of 400 μs; 80 loops; relaxation delay of 3.5 s; acquisition time of 2.73 s; TD of 64 k; and SW of 20.01 ppm.

Spectra were processed with a line broadening of 0.3 Hz and manually phased and baseline corrected using Topspin 1.3 software (Bruker, GmBH, Germany) and referenced to the α-glucose signal $\delta = 5.225$ ppm. The correction of peak positions (alignment) was performed using the correlation optimized warping algorithm COW and the icoshift algorithm implemented in Matlab (Matlab v. 8.1, Mathworks Inc.) [7,8]. The spectra-consistent 47 599 data points were normalized using the Probabilistic Quotient Normalization (PQN) method [9].

2.5. Multivariate data analysis for biomarker identification

Prior to chemometrics analysis, the data set was Pareto scaled. For primary visualization, distribution and clustering, the PCA model was applied. Next, the discriminant version of the Partial Least Squares regression (PLS-DA) with a 7-fold cross validation procedure (CV, 1/7 of the samples being excluded from calculations in each round) to determine variation between data sets was adopted.

The prediction performance of the PLS-DA models was estimated based on receiver operating characteristic (ROC) curves and area under curve (AUC) values. For this purpose, a perfcurve function from the Matlab statistical tool-box (Matlab v. 8.1, Mathworks, Inc.) was adopted. Specificity and sensitivity were determined according to sample class prediction using the 7-fold

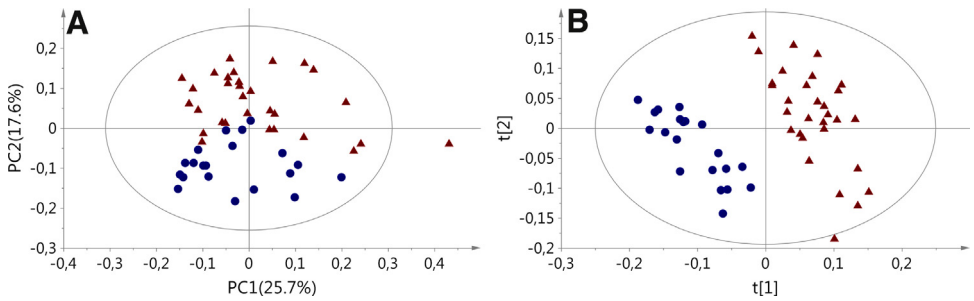

Fig. 3. The PCA model ((A); R^2X_{cum} = 0.731, Q^2_{cum} = 0.537) and PLS-DA model ((B); R^2X_{cum} = 0.528, Q^2cum = 0.946) for comparison HC and RAT group. Blue circles—healthy control; red tringles—patients after 3 months treatment. (For interpretation references to color in this figure legend, the reader is referred to the web version of this article.)

cross-validated predicted values of the fitted Y-predcv (implemented in SIMCA-P+ software) for observations in the model.

2.6. Statistical data analysis

The concentration of the metabolites based on ^{1}H NMR spectroscopy was calculated as relative signal integrals of the non-overlapping resonances (or a cluster of partly overlapping resonances). The metabolite resonances were identified according to assignments published in the literature and in on-line databases (Biological Magnetic Resonance Data Bank and Human Metabolome Data Base). For each metabolite, the percentage difference (PD) and relative standard deviation (RSD) were calculated using STATISTICA 10. The percentage difference was calculated based on the average values of relative signal integrals in each group. For each metabolite, the statistical significance based on Student's t test was calculated (p value less than 0.05).

2.7. Metabolite pathway analysis

To identify the most altered metabolic pathways, a set of significantly altered metabolites was used as the input for Metabolite Pathway Analysis (MetPA). The MetPA analysis was performed with a free on-line tool (http://www.msea.ca). Over-representation analysis (ORA) was used for comprehensive screening of affected pathways. P-values and false discovery rates (FDR) are reported [10].

3. Results

3.1. Response to treatment

Clinical data from 20 female Caucasian patients with RA treated with TNF-α inhibitors were analyzed. Among them, 80% were treated with ETA and 20% with ADA (Table 1). The mean DAS28 at the onset of biologic treatment was 6.84 ± 0.63 (range 5.24–8.05). No difference in DAS28 values (ETA—6.51 ± 0.66, ADA—6.54 ± 0.63, p = 0.1390) was detected between subgroups treated with different TNF-α inhibitors. The mean DAS28 after 24 weeks of treatment was 4.22 ± 1.10 (range 2.1–5.31). A moderate EULAR response was achieved in 55% of patients, while a good EULAR response was achieved in 20% of patients at 3 months.

3.2. Clustering of tested samples

The ^{1}H NMR-based metabolomics study was performed on 20 patients with rheumatoid arthritis: before and 3 months after induction of therapy with anti-TNF-α agents, and 30 healthy controls. The representative median spectrum of serum samples obtained from RA with assigned metabolites is shown in Fig. 1S.

To visualize the clustering of tested samples, a PCA model was constructed. The two first principal components (PCs) showed a distinct separation between the following groups (Fig. 2S(A)). Generally, the PC1 explained 25.5% of the total variance, and the PC2 explained 14.9%. Better classification and clear separation could be observed in the PLS-DA model (Fig. 2S(B)) with good model parameters (R^2X_{cum} = 0.635, Q^2_{cum} = 0.845) and statistically significant values by CV-ANOVA (less than 0.05).

3.3. Metabolomic basis of rheumatoid arthritis

Using the serum samples, the metabolic basis of rheumatoid arthritis was studied. For this purpose, groups of HC and RA were compared. The sample separation based on the PCA model is shown in Fig. 1(A).

The potential biomarkers were found using the PLS-DA model (Fig. 1(B)). The results showed that twelve metabolites were important for the discrimination of the groups HC and RA (Table 2). Note that valine, isoleucine, lactate, alanine, creatinine, GPC + APC and histidine were lower in RA, while 3-hydroxyisobutyrate, acetate, NAC, acetoacetate and acetone were higher.

The predictive ability and validation of the PLS-DA model were determined using the predictive set of samples.

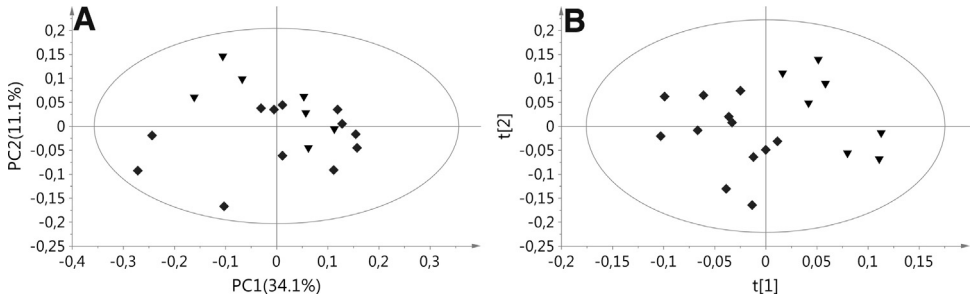

Fig. 4. The PCA model ((A); R^2X_{cum} = 0.554, Q^2_{cum} = 0.307) and PLS-DA model ((B); R^2X_{cum} = 0.534, Q^2_{cum} = 0.627) for comparison of patients with polymorphisms of *IL-17A*. Green diamonds—GG homozygosis; black inverted triangles—AA and GA genotypes. (For interpretation references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
The changes in relative integrals of serum metabolites.

Metabolite	Percentage difference			Relative standard deviation [%]		
	RA vs. HC	RAT vs. HC HCHCRAT	RA vs. RAT	HC	RA	RAT
L1	−7.5	−25.6[a]	18.1[a]	15.0	13.3	18.9
L2	7.5	5.7	1.8	28.8	24.8	23.8
Leucine	−4.3	11.9[a]	−16.2[a]	12.3	14.6	9.7
Valine	−17.2[a]	−7.1	−10.1	17.3	24.2	12.4
Isoleucine	−11.7[a]	−14.2[a]	2.5	14.4	18.8	9.4
3-Hydroxyisobutyrate	18.3[a]	−2.9	21.2[a]	23.9	32.7	18.4
L3	−2.6	−16.2[a]	13.5	27.0	25.5	24.4
Lactate	−26.5[a]	−15.0	−11.6	29.8	23.5	23.0
Alanine	−24.3[a]	−19.6[a]	−4.7	17.7	18.0	14.4
L4	2.0	−12.3	14.3	39.5	35.0	30.4
Lysine	−0.3	−7.8[a]	7.5[a]	11.1	12.2	9.1
Acetate	47.2[a]	67.6[a]	−22.1[a]	30.1	22.6	20.6
L5	1.4	−8.5[a]	9.9[a]	14.4	13.8	14.0
NAC	15.3[a]	16.8[a]	−1.5	9.9	11.1	11.2
Acetoacetate	55.1[a]	29.7[a]	26.5[a]	33.5	44.2	30.6
Acetone	17.4[a]	15.4[a]	2.0	11.5	23.0	9.8
Unk_1	−32.8	−46.7[a]	14.5	30.7	30.6	9.2
Citrate	4.0	9.6[a]	−5.6	11.1	10.9	6.6
Creatine	−3.0	−15.3[a]	12.3[a]	18.2	16.8	16.6
Creatinine	−11.5[a]	−17.6[a]	6.1	14.1	18.4	11.0
Choline	−1.4	5.8	−7.2	29.8	18.2	25.1
GPC + APC	−11.6[a]	−21.8[a]	10.3[a]	12.0	14.6	16.7
Betaine	−13.5	5.5	−19.0[a]	28.6	25.9	24.2
Glucose	6.7	2.3	4.4	12.4	11.6	9.3
L6	−1.8	−13.4	11.6	24.2	19.0	21.7
Tyrosine	−8.8	−34.5[a]	25.9	27.5	55.1	21.9
Histidine	−36.4[a]	−49.6[a]	13.9[a]	14.7	16.7	20.7
Phenylalanine	3.4	−48.7[a]	52.0[a]	16.6	42.5	26.2
Formate	60.7	138.7[a]	−98.8[a]	58.8	122.6	12.8

Percentage difference was calculated based on the average values of relative signal integrals in each group. The calculations were made from left to right.
[a] Statistically significant metabolites based on Student's *t* test.

According to the ROC curve (Fig. 1(C)) and the AUC values (model set = 0.893; prediction set = 1.000), the PLS-DA model showed excellent discriminant properties for comparing HC and RA (Fig. 3S).

3.4. Metabolomic monitoring of the rheumatoid arthritis treatment

In this study, the metabolomic serum approach was also employed for monitoring the rheumatoid arthritis treatment. For this purpose, the RA and RAT groups were compared.

The PCA model (Fig. 2(A)) showed a clear separation between RA and RAT according to the two first PCs. PC1 represented 27.1% of total variance, whereas PC2 represented 14.8%. For selection of the most important metabolites allowing the separation of RA and RAT, the PLS-DA model was constructed (Fig. 2(B)). Simultaneously, the ROC curve (Fig. 2(C)) and AUC values (model set = 0.964; prediction set = 1.000) showed excellent predictive properties for this comparison (Fig. 3S). The analysis of the relative concentration of metabolites revealed that L1, 3-hydroxyisobutyrate, lysine, L5, acetoacetate, creatine, GPC + APC, histidine and phenylalanine were elevated in RA, while leucine, acetate, betaine and formate were decreased.

When the HC group was compared to RAT both the PCA and PLS-DA models showed that patients after treatment did not move toward the healthy controls but rather formed a separate group (Fig. 3(A) and (B)) (Fig. 3S).

3.5. Metabolic changes related to the IL-17A polymorphism in patients with RA

In our previous study, the IL-17A (rs2275913, G-197A) polymorphism was found to affect RA progression and response to anti-TNF-α treatment. Interestingly, the separation of patients according to the *IL-17A* polymorphism (GG vs. GA, AA genotype carriers) can also be observed from the PCA and PLS-DA models (Fig. 4(A) and (B)). However, the number of tested samples should be higher for validation of this observation (Fig. 3S).

4. Discussion

4.1. Metabolic basis of RA

Rheumatoid arthritis is one of the most common autoimmune diseases, especially frequent among females, and causes systemic inflammation associated with joint lesions, leading to an uncomfortable life style for patients. In this study, we demonstrated the global metabolome obtained using ^{1}H NMR spectroscopy and showed the primary changes in metabolite profile related to RA.

The metabolite pathway analysis based on MetPA as well as the KEGG PATHWAY database (http://www.genome.jp/kegg/) indicated that the main differences between HC and RA are related to the network of propanoate metabolism; the synthesis and degradation of ketone bodies; valine, leucine and isoleucine degradation; glycolysis or gluconeogenesis; pyruvate metabolism; and glycerophospholipid metabolism (Fig. 4S). Yang et al. and Lauridsen et al. suggested that an elevated level of lactate could be one of the main biomarkers for the diagnosis of chronic inflammatory entities [11–13]. Indeed, lactate as a product of pyruvate is reported to be associated with a pathogenic role [11–13]. Moreover, the higher lactate relative concentration in RA may be related to low oxygen levels prevalent in inflammatory environments (increased NAC—*N*-acetylated glycoprotein) and the induction of hypoxia, promoting anaerobic respiration [11–16]. In opposite to above mentioned result we observed a reduced relative level of lactate among RA patients in comparison to HC subjects. The MetPA analysis (Fig. 4S) showed that a decreased relative concentration of lactate might be correlated with an increased overproduction of

ketone bodies, acetate, acetoacetate and acetone. Moreover, in this study, we observed in RA patients the degradation of ketogenic amino acids, valine and isoleucine and a higher relative level of 3-hydroxyisobutyrate (keto-metabolite).

Biosynthesis of the ketone bodies appears to be significantly combined with a limited energy source caused by low oxygen conditions in pathogenically changed tissues. However, inspection of Table 2 shows that the TCA cycle is intact; hence, energy demand should be assured by glucose utilization. The excess of ketone bodies in comparison to HC might reveal an inability in disposing ketone bodies or their overproduction by accelerated biochemical pathways. This finding might be a characteristic feature for RA patients. The decreased relative level of creatinine in both stages of RA and RAT, and only creatine in RAT, may suggest a lack of rapid conversion of creatine to creatinine during the progression of the disease, while after treatment, the relative levels of both metabolites are lower. The reduced relative level of derivatives of choline, namely, sn-glycero-3-phosphocholine (GPC) and acetylphosphocholine (APC), may indicate an extensive turnover of cell membranes (simultaneous degradation and biosynthesis) under inflammatory conditions [17].

4.2. Biomarkers for treatment efficacy

In this study, the metabolomics approach was used for assessment of the TNF-α inhibitor treatment. For this purpose, patients before and after three months of treatment were compared. We observed that the global metabolome measured by ^{1}H NMR spectroscopy was changed. Simultaneously, the potential biomarkers of efficacy of treatment were selected. Thirteen metabolites were statistically significant and determined the separation of the RA and RAT patients. The general overview of this set of metabolites showed that despite well-being during treatment, the patients are still far removed from the homeostasis represented by HC. However, there was no overlap with rheumatoid arthritis and inflammation in the global metabolome. There was a reduced relative level of ketone bodies, such as 3-hydroxyisobutyrate and acetoacetate, and an increased relative level of one ketogenic amino acid (leucine). Acetate was considerably higher after treatment. The persistent inflammatory process could be confirmed by the reduced relative level of LDL as a result of the activity of secretory phospholipase A2 that promotes the hydrolysis of phospholipids in LDL and VLDL. On the other hand, phenylalanine was decreased; thus, the immune system and inflammatory processes were not activated by the impairment of phenylalanine-4-hydroxylase [18]. The reduced relative level of GPC and APC, as well as the elevated concentration of betaine, indicate the turnover of phospholipids associated with reorganization of cell membranes. Finally, formate was increased (formed in the colonic lumen by intestinal bacteria, e.g., Enterobacteriaceae, in the process of unabsorbed carbohydrate fermentation) and may be related to rearrangement in the gut microbiome [19].

The comparison of RAT patients to HC subjects revealed that after treatment with TNF-α inhibitors, the RAT patients still showed signs of RA in the global metabolome. Thus, the question arises as to whether this treatment can restore one to health or if it merely temporarily disables the disease. Similar results were found by Lauridsen et al., where RA active group after treatment was almost of the same metabolic signature as group with RA in remission but still both group were different than HC [13]. However, the direction of changes in metabolites level as well the biomarker pool do not coincide with all our results.

4.3. Metabolic relationships with polymorphic variants of the IL-17A gene

There are no reports of the results of metabolomic and genomic studies in patients with RA. In this study, metabolomics tools were employed to discriminate patients with different *IL-17A* genotypes. Interestingly, both PCA and PLS-DA models showed significant differences between patients carrying the GG and AG/AA genotypes. These results are even more interesting because in our previous study we found that the GG homozygosity was associated with unfavorable outcome in female patients with RA. These patients more frequently presented with stage 4 RA and were characterized by more active disease after 3 months of therapy with TNF inhibitors [20]. Obviously due to a relatively small number of cases analyzed, more extended studies are required to validate our observations on the effect of genetic and metabolomic results and their prognostic value for females with RA. Nevertheless, these data concur with the previous observations, and suggest that the results of metabolomic (similarly to genetic) studies are of prognostic value for patients with RA.

5. Conclusion

Metabolomics may provide relevant biomarkers to improve diagnostic accuracy, define prognosis and predict and monitor treatment efficacy in RA.

Future advances of metabolomics in biomedical research will be particularly useful in the domain of rheumatic diseases, both for the identification of biomarkers and for new therapeutic targets.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.plantsci.2004.08.011.

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